Theoretical Study of Conformational Features of NAD+ and NADH Analogs: Protonated Nicotinamide and 1,4-Dihydronicotinamide

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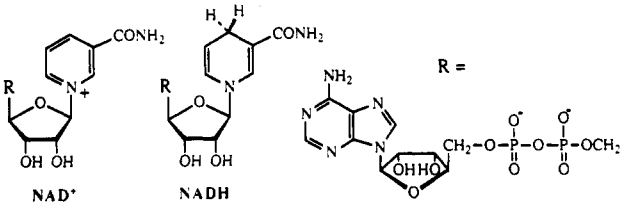
The conformations of nicotinamide (N^+) and 1,4-dihydronicotinamide (NH) have been studied by ab initio calculations at the MP2/6-31G*//6-31G* level. The amide group favors a cis conformation by about 1 kcal/mol for both N^+ and NH. The rotational barrier for cis to trans interconversion is about 4 and 7 kcal/mol for N^+ and NH, respectively. The amide group in the trans conformation is out of plane by about 20°. The 1,4-dihydronicotinamide ring is slightly puckered and adopts a boat conformation. The calculated ^{15}N equilibrium isotope effect for hydride transfer is small, in agreement with experimental observations.

Introduction

NAD^+/NADH coenzyme-dependent dehydrogenases catalyze alcohol to aldehyde or ketone interconversions.[1] The conformational features of the coenzymes are suggested to be related to the enzyme catalysis and stereospecificity.[2-6] For example, X-ray crystal structures of dehydrogenases with bound NAD^+ or NADH, as well as theoretical calculations, indicate that there is a correlation between enzyme stereospecificity and the ribose ring conformation with respect to the nicotinamide.[2,6] It has been suggested that the out-of-plane distortion of the nicotinamide amide is important for both the catalysis and stereochemistry of hydride transfer.[4,5]

In order to develop a quantitative understanding of the conformational features of the nicotinamide, we undertook a theoretical study of conformations of protonated nicotinamide (N^+) and 1,4-dihydronicotinamide (NH) with ab initio quantum mechanics. These calculations give much improved geometries and energetics of amide rotation from those found in previous studies.[7] We also calculated the ^{15}N equilibrium isotope effect for hydride transfer, for comparison with experimental observations.[8]



Theoretical Methods

The cis and trans conformations of N^+ and NH were fully optimized with the 3-21G and 6-31G* basis sets using the GAUSSIAN 90 program.[9] The transition structures for the cis to trans interconversions were calculated in the same manner for the two species. Each transition structure has one imaginary frequency corresponding to rotation. The ^{15}N isotope effects were calculated with 6-31G* vibrational frequencies which are scaled by 0.89.[10] The energies of stationary points were further evaluated with MP2/6-31G* calculations. The calculated energies of these structures are collected in Table I, and geometrical information is shown in Figures 1 and 2. Charges from a natural population analysis are given in Figure 1 for several atoms of interest.

Results and Discussion

The relative energies of the three conformations are quite basis set dependent (Table I) although the trends are similar at all levels. There is a larger preference for the cis conformation over the trans conformation with the 3-21G basis set. This is similar to the reported results of calculations for *N*-methyl analogs of N^+ and NH.[7] The cis preference is reduced significantly with higher levels of calculations. With correlation corrections at the MP2/6-31G* level, the trans conformation is less stable than the cis by only 0.9 and 1.0 kcal/mol for N^+ and NH, respectively. Experience with calculations on similar systems suggests that the actual energetics will be inter-

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Table I. Total Energies (-au) and Relative Energies (in Parentheses, kcal/mol) of the Cis and Trans Conformations and the Transition Structure for the Cis–Trans Conversion of Protonated Nicotinamide and 1,4-Dihydronicotinamide

nicotinamide	1, *cis*-N$^+$	3, *trans*-N$^+$	5, TS-N$^+$
3-21G//3-21G	412.538 04 (0.0)	412.533 95 (2.6)	412.524 23 (8.7)
6-31G*//3-21G	414.848 51 (0.0)	414.845 65 (1.8)	414.840 32 (5.1)
6-31G*//6-31G*	414.849 62 (0.0)	414.847 62 (1.3)	414.841 52 (5.1)
MP2/6-31G*/ /6-31G*	416.085 76 (0.0)	416.084 36 (0.9)	416.080 78 (3.1)
1,4-dihydro-nicotinamide	2, *cis*-NH	4, *trans*-NH	6, TS-NH
3-21G//3-21G	413.305 07 (0.0)	413.298 41 (4.2)	413.286 78 (11.5)
6-31G*//3-21G	415.615 23 (0.0)	415.610 69 (2.8)	415.601 70 (8.5)
6-31G*//6-31G*	415.616 34 (0.0)	415.613 37 (1.7)	415.603 85 (7.8)
MP2/6-31G*/ /6-31G*	416.864 41 (0.0)	416.862 80 (1.0)	416.853 26 (7.0)

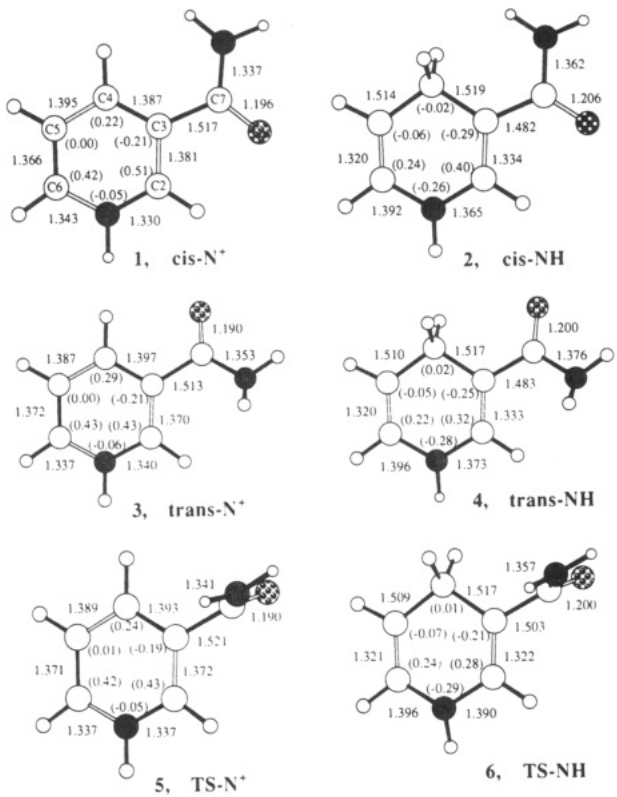

Figure 1. The 6-31G* geometries of cis and trans conformations and transition structure for the cis–trans conversion of protonated nicotinamide and 1,4-dihydronicotinamide. The values in parentheses are natural population charges at the ring carbons (with H atoms summed in).

mediate between the 6-31G* results and the MP2/6-31G* results.[11] Our best estimated relative energies are 0, 1, and 4 kcal/mol for *cis*-**N**$^+$, *trans*-**N**$^+$, and **TS-N**$^+$ and 0, 1.4, and 7.4 kcal/mol for *cis*-**NH**, *trans*-**NH**, and **TS-NH**, respectively. The cis conformation is stabilized by electrostatic interaction between the negatively charged amide oxygen and the positively charged C_2, as indicated qualitatively by natural population charges[12] which are shown in Figure 1. The large difference in charges at C_2 and C_4 is due to the attachment of C_2 to the electronegative N.

The cis conformation is observed in the X-ray structures of 1-methylnicotinamide picrate,[13] Li$^+$-NAD,[14] and *N*-benzyl-,[15] *N*-propyl-, and *N*-(methoxymethyl)-1,4-dihydronicotinamide,[16] in agreement with the calculations. However, the trans conformation is found in the X-ray structure of NAD$^+$ free acid and in most enzyme active sites.[17,18][13] The calculations indicate small energy differences of 1 kcal/mol between the cis and trans conformations, and solution, crystal, or enzymatic environments certainly provide interactions which can offset these small energy differences.

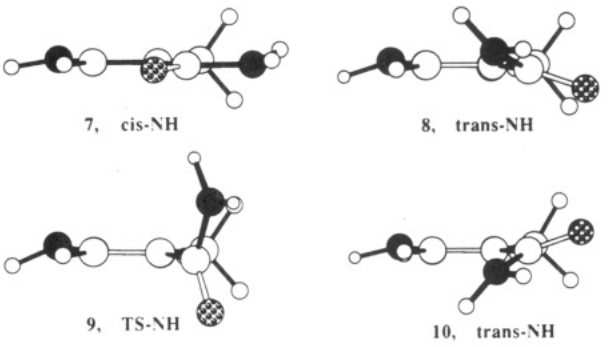

Figure 2. Side views of cis and trans conformations and the transition structure for the cis–trans conversion of 1,4-dihydronicotinamide (**7**–**9**) corresponding to structures **2**, **4**, and **6**. Structure **10** is the side view of another trans conformation with the NH_2 on the same side of the plane as the H(N), showing the carbonyl distortion in the same direction as ring puckering.

There is appreciable conjugation in **N**$^+$ which is interupted by rotation. This is even larger in **NH**, which is a vinylogous urea. This is also indicated in the C_3–C_7 bond lengths. In the transition structures the amide group is almost perpendicular to the pyridine ring. The dihedral angle of C_2—C_3—C_7=O is 88° in **5** and 86° in **6**. The π-conjugation is totally avoided in these structures. The C_3–C_7 bond is slightly longer for **N**$^+$ (**5** versus **1** and **3**), but is much longer in **NH** (**6** versus **2** and **4**).

The amide group is rotated substantially out-of-plane with respect to the pyridine in the trans conformations, as shown in Figure 2 for *trans*-**NH**. However, only small out-of-plane rotations are observed in the cis conforma-

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tions. In the trans conformers, there is substantial pyramidalization of the amide nitrogen.[19] The calculations by Gready et al. on the *N*-methyl analogs of **N**$^+$ and **NH** indicated that the potential energy surface is quite flat around the region where C_2–C_3–C_7–O = 180°.[7] Out-of-plane distortions in the trans conformations have small energetic consequences. Both rotation and NH_2 pyramidalization relieve steric interactions between the NH_2 group and C_2-H.

There have been many discussions about the activating effect of nicotinamide ring puckering on hydride transfer.[3,6,20] Similar to previous calculations, the 1,4-dihydronicotinamide ring is very slightly puckered into a boat conformation,[21] as shown in side views (Figure 2). There is significant pyramidalization at N_1. This phenomenon has been observed in the X-ray structure of NADH analog *N*-(methoxymethyl)-1,4-dihydronicotinamide.[16] In structure 8, the ring puckering and the carbonyl bond distortion are in the opposite directions. We also obtained a structure (**10**) with the carbonyl distorted in the same direction of the ring puckering. That structure has about the same degrees of carbonyl distortion and ring puckering and is only 0.2 kcal/mol less stable than 8. Therefore, the direction of amide rotation has little effect on the direction of the ring puckering.

Cleland et al. observed small ^{15}N equilibrium and kinetic isotope effects for the enzymatic reactions of 3-acetylpyridine adenine dinucleotide with cyclohexanol (LADH) and formate (FDH).[8] The calculated ^{15}N isotope effect of 1.015 for both cis and trans **N**$^+$/**NH** conversions is about 3 times that of the observed value of 1.0042 ± 0.0007. Considering the large pyramidalization at N_1 of **NH**, the observed and calculated ^{15}N isotope effects are quite small. This is because the nitrogen lone pair is largely conjugated with the C=C bonds, as indicated by short N_1–C_2 and N_1–C_6 bond lengths.

Why does the amide group adopt the trans conformation in most of the enzyme active sites even though the cis conformation is intrinsically more favorable? One possible answer is that the trans conformation is more reactive due to interactions which develop in the transition state for hydride transfer.[22-24] This will be the subject of future studies.

Conclusion

Both protonated nicotinamide (**N**$^+$) and 1,4-dihydronicotinamide (**NH**) favor cis conformations; the amide rotational barriers (cis to trans) for the two species are about 4 and 7–8 kcal/mol, respectively; these features should be useful for force-field studies of NAD$^+$ and NADH binding in enzyme active sites; the amide group in the trans conformations is rotated out-of-plane by 20–30°; there is a small ring puckering for **NH**; and despite the significant pyramidalization of N_1 in **NH**, the ^{15}N equilibrium isotope effect is small.

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